DEVICE AND METHOD FOR DETERMINING ANALYTE LEVELS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This is a continuation-in-part of Serial No. 09/447,227, filed November 22, 1999, which is a divisional of Serial No. 08/811,473, filed March 4, 1997, now U.S. Patent No.: 6,001,067.

FIELD OF THE INVENTION

[0002] The present invention relates generally to devices and methods for determining analyte levels, and, more particularly, to implantable devices and methods for monitoring glucose levels in a biological fluid.

BACKGROUND OF THE INVENTION

[0003] The continuous measurement of substances in biological fluids is of interest in the control and study of metabolic disorders. Electrode systems have been developed for this purpose whereby an enzyme-catalyzed reaction is monitored (*e.g.*, by the changing concentrations of reactants or products) by an electrochemical sensor. In such electrode systems, the electrochemical sensor comprises an electrode with potentiometric or amperometric function in close contact with a thin layer containing an enzyme in dissolved or insoluble form. Generally, a semipermeable membrane separates the thin layer of the electrode containing the enzyme from the sample of biological fluid that includes the substance to be measured.

[0004] Electrode systems that include enzymes have been used to convert amperometrically inactive substances into reaction products that are amperometrically active. For example, in the analysis of blood for glucose content, glucose (which is relatively inactive amperometrically) may be catalytically converted by the enzyme glucose oxidase in the presence of oxygen and water to gluconic acid and hydrogen peroxide. Tracking the concentration of glucose is thereby possible since for every glucose molecule reacted a proportional change in either oxygen or hydrogen peroxide sensor current will occur [U.S. Pat. Nos. 4,757,022 and 4,994,167 to Shults *et al.*, both of which are hereby incorporated by reference]. Hydrogen peroxide is anodically active and produces a current that is proportional to the concentration of hydrogen peroxide. [Updike *et al.*, Diabetes Care, 11:801-807 (1988)].

[0005] Despite recent advances in the field of implantable glucose monitoring devices, presently used devices are unable to provide data safely and reliably for long periods of time (*e.g.*, months or years) [*See, e.g.*, Moatti-Sirat *et al.*, Diabetologia 35:224-30 (1992)]. For example, Armour *et al.*, Diabetes 39:1519-26 (1990), describes a miniaturized sensor that is placed intravascularly, thereby allowing the tip of the sensor to be in continuous contact with the blood. Unfortunately, probes that are placed directly into the vasculature put the recipient at risk for thrombophlebosis, thromboembolism, and thrombophlebitis.

[0006] Currently available glucose monitoring devices that may be implanted in tissue (*e.g.*, subcutaneously) are also associated with several shortcomings. For example, there is no dependable flow of blood to deliver sample to the tip of the probe of the implanted device. Similarly, in order to be effective, the probe must consume some oxygen and glucose, but not

enough to perturb the available glucose which it is intended to measure; subcutaneously implanted probes often reside in a relatively low oxygen environment in which oxygen or glucose depletion zones around the probe tip may result in erroneously low measured glucose levels. In addition, implantable devices that utilize electrode sensors require membranes of the appropriate composition to protect the sensor from harsh *in vivo* environmental conditions. Current membrane technology has allowed the development of a single structural membrane that performs the same functions that previously required multiple membranes. However, these single membranes have been observed to delaminate and thus prevent accurate long-term glucose monitoring. Finally, the probe may be subject to "motion artifact" because the device is not adequately secured to the tissue, thus contributing to unreliable results. Partly because of these limitations, it has previously been difficult to obtain accurate information regarding the changes in the amounts of analytes (*e.g.*, whether blood glucose levels are increasing or decreasing); this information is often extremely important, for example, in ascertaining whether immediate corrective action is needed in the treatment of diabetic patients.

[0007] There is a need for a device that accurately and continuously determines the presence and the amounts of a particular analyte, such as glucose, in biological fluids. The device should be easy to use, be capable of accurate measurement of the analyte over long periods of time, and should not readily be susceptible to motion artifact.

SUMMARY OF THE INVENTION

[0008] The present invention relates generally to devices and methods for determining analyte levels, and, more particularly, to implantable devices and methods for monitoring glucose levels in a biological fluid.

[0009] In one aspect of the present invention, an implantable device for measuring an analyte in a biological fluid is provided, which includes the following: a housing including an electronic circuit; and a sensor operably connected to the electronic circuit of the housing, the sensor including i) a member for determining the amount of glucose in a biological sample ii) a bioprotective membrane, the bioprotective membrane positioned more distal to the housing than the glucose determining member and substantially impermeable to macrophages, and iii) an angiogenic layer, the angiogenic layer positioned more distal to the housing than the bioprotective membrane.

[0010] The present invention further encompasses a method of monitoring glucose levels, the method including the steps of providing a host, and an implantable device as described above and implanting the device in the host under conditions such that the device measures glucose for a period exceeding 360 days.

[0011] In one embodiment of this aspect, the invention encompasses a method of measuring glucose in a biological fluid that includes the steps of providing a host, and an implantable device as provided above, wherein the glucose determining member of the

implantable device is capable of continuous glucose sensing, and implanting the device in the host.

Definitions

[0012] In order to facilitate an understanding of the present invention, a number of terms are defined below.

[0013] The term "accurately" means, for example, 95% of measured values within 25% of the actual value as determined by analysis of blood plasma, preferably within 15% of the actual value, and most preferably within 5% of the actual value. Alternatively, "accurately" means that 85% of the measured values fall into the A and B regions of a Clarke error grid, or preferably 90%, or most preferably 95% of the measured values fall into these regions. It is understood that like any analytical device, calibration, calibration validation and recalibration are required for the most accurate operation of the device.

[0014] The term "analyte" refers to a substance or chemical constituent in a biological fluid (*e.g.*, blood or urine) that can be analyzed. A preferred analyte for measurement by the devices and methods of the present invention is glucose.

[0015] The terms "sensor interface," "sensor means," "sensor" and the like refer to the region of a monitoring device responsible for the detection of a particular analyte. For example, in some embodiments of a glucose monitoring device, the sensor interface refers to that region wherein a biological sample (*e.g.*, blood or interstitial fluid) or a portion thereof contacts

(directly or after passage through one or more membranes or layers) an enzyme (*e.g.*, glucose oxidase); the reaction of the biological sample (or portion thereof) results in the formation of reaction products that allow a determination of the glucose level in the biological sample. In preferred embodiments of the present invention, the sensor means comprises an angiogenic layer, a bioprotective layer, an enzyme layer, and an electrolyte phase (*i.e.*, a free-flowing liquid phase comprising an electrolyte-containing fluid [described further below]). In some preferred embodiments, the sensor interface protrudes beyond the plane of the housing.

[0016] The term "tissue interface" refers to that region of a monitoring device that is in contact with tissue.

[0017] The terms "operably connected," "operably linked," and the like refer to one or more components being linked to another component(s) in a manner that allows transmission of, *e.g.*, signals between the components. For example, one or more electrodes may be used to detect the amount of analyte in a sample and convert that information into a signal; the signal may then be transmitted to electronic circuit means (*i.e.*, the electrode is "operably linked" to the electronic circuit means), which may convert the signal into a numerical value in the form of known standard values.

[0018] The term "electronic circuit means" or "electronic circuit" refers to the electronic circuitry components of a biological fluid measuring device required to process information obtained by a sensor means regarding a particular analyte in a biological fluid, thereby providing data regarding the amount of that analyte in the fluid. U.S. Pat. No. 4,757,022 to Shults *et al.*,

previously incorporated by reference, describes suitable electronic circuit means (*see, e.g.*, FIG. 7); of course, the present invention is not limited to use with the electronic circuit means described therein. A variety of circuits are contemplated, including but not limited to those circuits described in U.S. Pat. Nos. 5,497,772 and 4,787,398, hereby incorporated by reference.

[0019] The terms "angiogenic layer," "angiogenic membrane," and the like refer to a region, membrane, etc. of a biological fluid measuring device that promotes and maintains the development of blood vessels microcirculation around the sensor region of the device. As described in detail below, the angiogenic layer of the devices of the present invention may be constructed of membrane materials alone or in combination such as polytetrafluoroethylene, hydrophilic polyvinylidene fluoride, mixed cellulose esters, polyvinylchloride, and other polymers including, but not limited to, polypropylene, polysulfone, and polymethylmethacrylate.

[0020] The phrase "positioned more distal" refers to the spatial relationship between various elements in comparison to a particular point of reference. For example, some embodiments of a biological fluid measuring device comprise both a bioprotective membrane and an angiogenic layer/membrane. If the housing of the biological fluid measuring device is deemed to be the point of reference and the angiogenic layer is positioned more distal to the housing than the bioprotective layer, then the bioprotective layer is closer to the housing than the angiogenic layer.

[0021] The terms "bioprotective membrane," "bioprotective layer," and the like refer to a semipermeable membrane comprised of protective biomaterials of a few microns thickness or

more that are permeable to oxygen and glucose and are placed over the tip of the sensor to keep the white blood cells (*e.g.*, tissue macrophages) from gaining proximity to and then damaging the enzyme membrane. In some embodiments, the bioprotective membrane has pores (typically from approximately 0.1 to approximately 1.0 micron). In preferred embodiments, a bioprotective membrane comprises polytetrafluoroethylene and contains pores of approximately 0.4 microns in diameter. Pore size is defined as the pore size provided by the manufacturer or supplier.

[0022] The phrase "substantially impermeable to macrophages" means that few, if any, macrophages are able to cross a barrier (*e.g.*, the bioprotective membrane). In preferred embodiments, fewer than 1% of the macrophages that come in contact with the bioprotective membrane are able to cross.

[0023] The phrase "material for securing said device to biological tissue" refers to materials suitable for attaching the devices of the present invention to, the fibrous tissue of a foreign body capsule. Suitable materials include, but are not limited to, poly(ethylene terephthalate). In preferred embodiments, the top of the housing is covered with the materials in the form of surgical grade fabrics; more preferred embodiments also contain material in the sensor interface region (*see* FIG. 1B).

[0024] The phrase "member for determining the amount of glucose in a biological sample" refers broadly to any mechanism (*e.g.*, enzymatic or non-enzymatic) by which glucose can be quantitated. For example, some embodiments of the present invention utilize a membrane that contains glucose oxidase that catalyzes the conversion of glucose to gluconate: Glucose + O_2

= Gluconate + H_2O_2. Because for each glucose molecule converted to gluconate, there is a proportional change in the co-reactant O_2 and the product H_2O_2, one can monitor the current change in either the co-reactant or the product to determine glucose concentration.

[0025] The phrase "apparatus for transmitting data to a location external to said device" refers broadly to any mechanism by which data collected by a biological fluid measuring device implanted within a subject may be transferred to a location external to the subject. In preferred embodiments of the present invention, radiotelemetry is used to provide data regarding blood glucose levels, trends, and the like.

[0026] The terms "radiotelemetry," "radiotelemetric device," and the like refer to the transmission by radio waves of the data recorded by the implanted device to an *ex vivo* recording station (*e.g.*, a computer), where the data is recorded and, if desired, further processed (*see, e.g.*, U.S. Pat. Nos. 5,321,414 and 4,823,808, hereby incorporated by reference; PCT Pat. Publication WO 94/22367).

[0027] The term "host" refers to both humans and animals.

[0028] The phrase "continuous glucose sensing" refers to the period in which monitoring of plasma glucose concentration is continuously carried out. More specifically, at the beginning of the period in which continuous glucose sensing is effected, the background sensor output noise diminishes and the sensor output stabilizes (*e.g.*, over several days) to a long-term level

reflecting adequate microcirculatory delivery of glucose and oxygen to the tip of the sensor (*see* FIG. 2).

[0029] The term "filtrate layer" refers to any permeable membrane that is able to limit molecules from passing through the membrane based on their properties including molecular weight. More particularly, the resistance layer, interference layer and bioprotective membrane are examples of layers that can function as filtrate layers, depending on the materials from which they are prepared. These layers can control delivery of analyte to a sensing means. Furthermore, these layers can reduce a number of undesirable molecular species that may otherwise be exposed to the sensor for detection and provide a controlled sample volume to the analyte sensing means.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] FIG. 1A depicts a cross-sectional drawing of one embodiment of an implantable analyte measuring device of the present invention.

[0031] FIG. 1B depicts a cross-sectional exploded view of the sensor interface dome of FIG. 1A.

[0032] FIG. 1C depicts a cross-sectional exploded view of the electrode-membrane region of FIG. 1B detailing the sensor tip and the functional membrane layers.

[0033] FIG. 2 graphically depicts glucose levels as a function of the number of days post-implant.

[0034] FIG. 3 is a graphical representation of the number of functional sensors versus time (i.e. weeks) comparing control devices including only a cell-impermeable domain ("Control"), with devices including a cell-impermeable domain and a barrier-cell domain ("Test").

[0035] FIG. 4A is a photograph of an intact composite bioprotective/angiogenic membrane after implantation in a dog for 137 days.

[0036] FIG. 4B is a photograph of a delaminated ePTFE bilayer membrane after implantation in a dog for 125 days.

DETAILED DESCRIPTION OF THE INVENTION

[0037] The present invention relates generally to devices and methods for determining analyte levels, and, more particularly, to implantable devices and methods for monitoring glucose levels in a biological fluid. In a preferred embodiment, the device and methods of the present invention are used to determine the level of glucose in a host, a particularly important measurement for individuals having diabetes.

[0038] Although the description that follows is primarily directed at glucose monitoring devices and methods for their use, the devices and methods of the present invention are not

limited to glucose measurement. Rather, the devices and methods may be applied to detect and quantitate other analytes present in biological fluids (including, but not limited to, amino acids and lactate), especially those analytes that are substrates for oxidase enzymes [*see, e.g.*, U.S. Pat. No. 4,703,756 to Gough *et al.*, hereby incorporated by reference]. Moreover, the devices and methods of the present invention may be utilized to present components of biological fluids to measurement methods which are not enzyme-based, including, but not limited to, those based on surface plasmon resonance, surface acoustic waves, optical absorbance in the long wave infrared region, and optical rotation of polarized light.

[0039] For example, surface plasmon resonance sensors that analyze a region within less than one wavelength of analysis light near the flat surface of the sensor have been described (*See* U.S. Pat. No. 5,492,840). These sensors have been used, for example, in the study of immunochemistry and other surface bound chemical reactions (Jonsson et al., Annales de Biologies Clinique 51(10:19, 1993). This type of sensor may be incorporated into the implantable device of the present invention for the detection of a number of different analytes including glucose. One skilled in the art would recognize that the surface plasmon resonance sensor is an optical sensor and that the implantable device of the present invention may further include a source of coherent radiation (e.g. a laser operating in the visible or near infrared).

[0040] In one application, referred to here as a consumptive approach, an enzyme that consumes the analyte producing a detectable product is immobilized on the sensor in the filtrate layer. When the enzyme consumes the analyte, the reaction products diffuse away from the enzyme at a rate dependent on the permeability of the layers distal to the enzyme layer. As a

result, reaction products will accumulate at a higher concentration near the sensor, within one wavelength of analysis light, where they may be detected and measured. One example of such a system that detects the presence of glucose would immobilize a glucose oxidase enzyme layer on the sensor surface.

[0041] The layers of the present invention play an important role in the effective operation and function of this type of sensor. In particular, the angiogenic layer assures a constant supply of analyte from the tissues of the subject, the bioprotective membrane protects the underlying layers from cellular attack, the resistance layer controls the rate of delivery of analyte and the filtrate layer performs many functions including; providing a low molecular weight filtrate, reducing the number of undesirable molecular species available to the sensor for detection and providing a controlled volume of sample for detection by the sensor. As mentioned above, the bioprotective membrane, resistance layer and interference layer can function as filtrate layers. For example, it is well within the contemplation of the present invention that the bioprotective membrane can be made of a material that is able to exclude certain molecules from passing through the membrane based on their size.

[0042] One skilled in the art would recognize that the reaction kinetics associated with each type of enzyme that may be selected for use with this sensor is unique. However, in general, if an excess of enzyme is provided, the enzyme turnover rate is proportional to the flux of analyte to the enzyme and independent of the enzyme concentration. Therefore, the actual analyte concentration may be calculated utilizing the diffusion rate of the detectable analyte across the bioprotective resistance layers.

[0043] In another application, referred to here as a non-consumptive approach, an analyte-binding compound is provided on the surface plasmon resonance sensor surface within one wavelength of analysis light. This compound reversibly binds, but does not consume, the analyte. In this application, the analyte moves reversibly onto and off of attachment sites on the binding compound. This reaction provides a steady state condition for bound and unbound analyte that may be quantitated and analyte concentration mathematically calculated. One skilled in the art would recognize that the reaction kinetics associated with binding and release of the analyte is unique for each type of binding compound selected. Examples of such a system that detects the presence of glucose provide a binding compound comprised of conconavalin A or a wide range of borate containing compounds (See U.S. Pat. No. 6,011,985).

[0044] Since this is a chemical equilibrium-based approach, a filtrate layer is not necessarily required to maintain an analyte concentration near the sensor. However, such a membrane would still be desired to reduce the number of undesirable molecular species available to the analyte-binding layer. Preferably, the bioprotective layer is thin to allow rapid sensor equilibration to changes in analyte levels. As described above, one skilled in the art would recognize that the function of the filtrate layer could be incorporated into the bioprotective membrane by selection of the appropriate molecular exclusion, such as exclusion by molecular weight, if desired.

[0045] A variety of materials may be utilized to construct a combination angiogenic/bioprotective membrane, many of which are described below under the angiogenic layer and bioprotective membrane headings. Preferably, this combination membrane is ePTFE

embedded in a layer of PVP containing urethane hydrogel. However, any material that performs a similar function as the PVP containing polyurethane hydrogel could be substituted.

[0046] In either application, consumptive or non-consumptive, one skilled in the art would recognize that the response time of the sensor is subject to Fick's law of diffusion. More specifically, sensors with thick membrane layers or that have low analyte diffusivity will respond slower to change in analyte concentration than sensors with thin membranes or that have high analyte diffusivity. Consequently, reasonable optimization experimentation with the membrane and layers would be required to meet various use requirements.

[0047] One skilled in the art would further recognize that the consumptive or non-consumptive approaches of the previous example could be applied to additional sensor modalities as follows:

[0048] 1. Another sensor that may be incorporated into the device of the present invention that has been previously described is a surface acoustic wave sensor (See U.S. Pat. No. 5,932,953). This sensor, also referred to as a bulk-acoustic wave piezoelectric resonator, typically includes a planar surface of piezoelectric material with two respective metal layers bonded on opposite sides that form the electrodes of the resonator. The two surfaces of the resonator are free to undergo vibrational movement when the resonator is driven by a signal within the resonance band of the resonator. One of these surfaces is adapted to provide reversible binding sites for the analyte being detected. The binding of the analyte on the surface of the resonator alters the resonant characteristics of the resonator and changes in the resonant

characteristics may be detected and interpreted to provide quantitative information regarding the analyte concentration.

[0049] 2. Another sensor that may be incorporated into the device of the present invention is an optical absorbance sensor (See U.S. Pat. No. 6,049,727). This sensor utilizes short to medium wavelength infrared light that is passed through a sample with the unabsorbed infrared light being monitored by an optical detector.

[0050] Previously developed methods for analysis of analytes such as glucose in tissues and blood have been relatively unsuccessful for two reasons, interference from other chemicals present in the complex biological sample and signal variation due to poor control of sample volume. These problems may be solved by providing a low molecular weight filtrate of biological fluid in a controlled volume of sample to the sensor. In one system of the present invention, biological analyte is provided to the sensor through the angiogenic layer. This analyte is then filtered through the bioprotective membrane to produce a desirable filtrate. Alternatively, a third filtrate layer, such as an interference layer, may be utilized having specific filtration properties to produce the desired filtrate. The three-dimensional structure of the bioprotective membrane and/or other filtrate layers is utilized to define and stabilize the sample volume. One skilled in the art would recognize that any material that provides a low molecular weight filtrate to the sensor in a controlled volume might be utilized. Preferably, this material is polyurethane.

[0051] The sensor may be enhanced by partial metallization of the distal side of the filtrate producing material that would serve to isolate by reflection the optical signal to the space

within the filtrate region directly adjacent to the sensor. This metal film may be a durable metal including, but not restricted to, gold or platinum and may be vacuum deposited onto the filtrate producing material.

[0052] One skilled in the art would recognize that the optical absorbance sensor requires a source of short to medium wavelength infrared light. Consequently, the implantable device of the present invention would further include a source of infrared radiation and an optical detector.

[0053] 3. Another sensor that may be incorporated into the device of the present invention that has been previously described is a polarized light optical rotation sensor (See U.S. Pat. No. 5,209,231). This sensor may be used to detect an analyte that rotates polarized light such as glucose. In particular, glucose concentrations in biological fluids in the range of 0.05 to 1.00% w/v may be detected and quantitated. Normal non-diabetic subjects generally have biological glucose concentrations ranging from 0.07 to 0.12% w/v.

[0054] In this type of sensor, the optical detector receives polarized light passed through a biological sample and then further through a polarizing filter. The optical activity of an analyte in the sample rotates the polarized light in proportion to its concentration. Unfortunately, accurate measurements of glucose in complex biological samples has proven difficult because of the optical activity of interfering substances and poor control of sample volume. These problems may be solved by providing a low molecular weight filtrate of biological fluid in a controlled volume to the sensor. The present invention meets this criterion by providing a continuous supply of biological glucose to the sensor through the angiogenic layer that is filtered through a

bioprotective membrane and/or a filtrate layer as described previously for the optical absorbance sensor. One skilled in the art would recognize that any material that provides a low molecular weight filtrate to the sensor in a controlled geometry might be utilized. Preferably, this material is polyurethane. In addition, one skilled in the art would recognize that the polarized light optical rotation sensor requires a source of polarized light. Consequently, the implantable device of the present invention would further include a source of polarized radiation.

[0055] 4. Another sensor that may be incorporated into the device of the present invention that has been previously described is a fluorescence sensor (See U.S. Pat. No. 5,341,805). The invention of Colvin provides a method for incorporating an ultraviolet light source and fluorescent sensing molecules in an implantable device. However, Colvin does not describe how the sensor would survive harsh *in vivo* environmental conditions, how the device would be functionally integrated into body tissues or how a continuous supply of glucose would be maintained for detection by the sensor. These problems may be solved by providing a low molecular weight filtrate of biological fluid in a controlled volume to the sensor.

[0056] In this example, a continuous supply of biological glucose passes to the sensor through the angiogenic layer that prevents isolation of the sensor by the body tissue. The glucose is then filtered through the bioprotective membrane to produce a desirable filtrate having fewer interfering molecules and to protect the sensor from *in vivo* environmental conditions. Alternatively, a filtrate layer may be utilized having specific filtration properties to produce the desired filtrate. The three-dimensional structure of the bioprotective membrane and/or filtrate layer also provides stabilized sample volume for detection by the sensor.

[0057] One skilled in the art would recognize that a fluorescence sensor requires a source of light. Consequently, the implantable device of the present invention would further comprise a source of radiation, as well as fluorescent sensing molecules to detect the presence of analyte.

I. Nature of the Foreign Body Capsule

[0058] Devices and probes that are implanted into subcutaneous tissue will almost always elicit a foreign body capsule (FBC) as part of the body's response to the introduction of a foreign material. Therefore, implantation of a glucose sensor results in an acute inflammatory reaction followed by building of fibrotic tissue. Ultimately, a mature FBC including primarily a vascular fibrous tissue forms around the device (Shanker and Greisler, Inflammation and Biomaterials in Greco RS, ed. Implantation Biology: The Host Response and Biomedical Devices, pp68-80, CRC Press (1994)).

[0059] Although fluid is frequently found within the capsular space between the sensor and the capsule, levels of analytes (*e.g.*, glucose and oxygen) within the fluid often do not mimic levels in the body's vasculature, making accurate measurement difficult.

[0060] In general, the formation of a FBC has precluded the collection of reliable, continuous information, reportedly because of poor vascularization, the composition of a FBC has prevented stabilization of the implanted device, contributing to motion artifact that renders unreliable results. Thus, conventionally, it has been the practice of those skilled in the art to attempt to minimize FBC formation by, for example, using a short-lived needle geometry or

sensor coatings to minimize the foreign body reaction ("Biosensors in the Body" David M. Fraser, ed.;1997 pp 117-170. Wiley & Sons Ltd., West Sussex, England),

[0061] In contrast to the prior art, the teachings of the present invention recognize that FBC formation is the dominant event surrounding long term implantation of any sensor and must be orchestrated to support rather than hinder or block sensor performance. For example, sensors often do not perform well until the FBC has matured sufficiently to provide ingrowth of well-attached tissue bearing a rich supply of capillaries directly to the surface of the sensor. With reference to Figure 2, stabilization of device function generally occurs between about 2 and 8 weeks depending on the rate of healing and formation of new capillaries. In some cases, devices are functional from the time of implant, and sometimes it may take as long as 12 weeks.. However, the majority of devices begin functioning between weeks 2 and 8 after implantation. This maturation process, when initiated according to the present invention, is a function of biomaterial and host factors that initiate and modulate angiogenesis, and promote and control fibrocyte ingrowth. The present invention contemplates the use of particular materials to promote angiogenesis adjacent to the sensor interface (also referred to as the electrode-membrane region, described below) and to anchor the device within the FBC.

II. The Implantable Glucose Monitoring Device of the Present Invention

[0062] The present invention contemplates the use of a unique micro-geometry at the sensor interface of an implantable device. Moreover, the present invention contemplates the use of materials covering all or a portion of the device to assist in the stabilization of the device following implantation. However, it should be pointed out that the present invention does not

require a device comprising particular electronic components (*e.g.*, electrodes, circuitry, etc). Indeed, the teachings of the present invention can be used with virtually any monitoring device suitable for implantation (or subject to modification allowing implantation); suitable devices include, but are not limited, to those described in U.S. Pat. Nos. 6,001,067 to Shults *et al.*; U.S. Pat. No. 4,703,756 to Gough *et al.*, and U.S. Pat. No. 4,431,004 to Bessman *et al.*; the contents of each being hereby incorporated by reference, and Bindra *et al.*, Anal. Chem. 63:1692-96 (1991).

[0063] In the discussion that follows, an example of an implantable device that includes the features of the present invention is first described. Thereafter, the specific characteristics of, for example, the sensor interface contemplated by the present invention will be described in detail.

[0064] Generally speaking, the implantable devices contemplated for use with the present invention are cylindrical or oval shaped; of course, devices with other shapes may also be used with the present invention. The sample device includes a housing composed of radiotransparent ceramic. FIG. 1A depicts a cross-sectional drawing of one embodiment of an implantable measuring device. Referring to FIG. 1A, the cylindrical device includes a ceramic body 1 and ceramic head 10 houses the sensor electronics that include a circuit board 2, a microprocessor 3, a battery 4, and an antenna 5. Furthermore, the ceramic body 1 and head 10 possess a matching taper joint 6 that is sealed with epoxy. The electrodes are subsequently connected to the circuit board via a socket 8.

[0065] As indicated in detail in FIG. 1B, three electrodes protrude through the ceramic head 10, a platinum working electrode 21, a platinum counter electrode 22 and a silver/silver chloride reference electrode 20. Each of these is hermetically brazed 26 to the ceramic head 10 and further affixed with epoxy 28. The sensing region 24 is covered with the sensing membrane described below and the ceramic head 10 contains a groove 29 so that the membrane may be affixed into place with an o-ring.

[0066] In a preferred embodiment, the device is cylindrical, as shown in FIG. 1A, and is approximately 1 cm in diameter, and 5.5 cm long. The sensing region is situated at one extreme end of the cylinder. The sensor region includes a dome onto which the sensing membranes are attached.

[0067] Maintaining the blood supply near an implanted foreign body like an implanted analyte-monitoring sensor requires stable fixation of FBC tissue on the surface of the foreign body. This can be achieved, for example, by using capsular attachment (anchoring) materials (*e.g.*, those materials that includes the sensor interface and tissue anchoring layers) developed to repair or reinforce tissues, including, but not limited to, polyester (DACRON®; DuPont; poly(ethylene terephthalate)) velour, expanded polytetrafluoroethylene (TEFLON®; Gore), polytetrafluoroethylene felts, polypropylene cloth, and related porous implant materials. In a preferred embodiment, porous silicone materials are used for anchoring the device. In another embodiment, non-woven polyester fibers are used for anchoring the device. Tissue tends to aggressively grow into the materials disclosed above and form a strong mechanical bond (*i.e.*,

tissue anchoring); this fixation of the implant in its capsule is essential to prevent motion artifact or disturbance of the newly developed capillary blood supply.

[0068] In a preferred embodiment, the anchoring material is attached directly to the body of the device. In the case of non-woven polyester fibers, they may be sutured into place by rolling the material onto the circumferential periphery of the device and further encircling the membrane with PTFE sutures and tying the sutures to hold the membrane in place. In another preferred embodiment, porous silicone is attached to the surface of the cylindrical device using medical grade silicone adhesive. In either case, the material may be further held in place by an o-ring (Fig. 1B).

[0069] As shown in Fig. 1A, the interior of the housing contains one or more batteries 4 operably connected to an electronic circuit means (*e.g.*, a circuit board 2), which, in turn, is operably connected to at least one electrode (described below); in another embodiment, at least two electrodes are carried by the housing. In a preferred embodiment, three electrodes are used. Any electronic circuitry and batteries that render reliable, continuous, long-term (*e.g.*, months to years) results may be used in conjunction with the devices of the present invention.

[0070] The housing of the devices of the present invention preferably contain a biocompatible ceramic material. A preferred embodiment of the device contains a radiofrequency transmitter and antenna within the body of the ceramic device. Ceramic materials are radiotransparent and, therefore, are preferred over metals that are radioopaque. Ceramic materials are preferred over plastic materials (which may also be radiotransparent)

because they are more effective than plastics at preventing water penetration. In one embodiment of the invention, the ceramic head and body are connected at an approximately 0.9 cm long taper joint sealed with epoxy. In other embodiments, the head and body may be attached by sealing with metals to produce a completely hermetic package.

[0071] FIG. 1C depicts a cross-sectional exploded view of the electrode-membrane region 24 set forth in FIG. 1B detailing the sensor tip and the functional membrane layers. As depicted in FIG. 1C, the electrode-membrane region includes several different membrane layers, the compositions and functions of which are described in detail below. The top ends of the electrodes are in contact with the electrolyte phase 30, a free-flowing fluid phase. The electrolyte phase is covered by the sensing membrane 32 that contains an enzyme, *e.g.*, glucose oxidase, and several functional polymer layers (as described below). In turn, a composite bioprotective/angiogenic membrane 33 covers the sensing membrane 32 and serves, in part, to protect the sensor from external forces that may result in environmental stress cracking of the sensing membrane 32.

[0072] In one preferred embodiment of the inventive device, each of the membrane layers is affixed to the ceramic head 10 in FIGS. 1A and 1B by an o-ring. The o-ring may be formed of fluoroelastomer.

[0073] The present invention contemplates the construction of the membrane layers of the sensor interface region using standard film coating techniques. This type of membrane fabrication facilitates control of membrane properties and membrane testing.

III. The Sensor Interface Region

[0074] As mentioned above and disclosed in FIG. 1C, in a preferred embodiment, the sensor interface region includes several different layers and membranes that cover the electrodes of an implantable analyte-measuring device. The characteristics of these layers and membranes are now discussed in more detail. The layers and membranes prevent direct contact of the biological fluid sample with the electrodes, while permitting selected substances (*e.g.*, analytes) of the fluid to pass therethrough for electrochemical reaction with the electrodes.

[0075] Measurement of analyte in a filtrate of biological fluid samples has been shown to be preferred over direct measurement of analyte in biological fluid in order to minimize effects of interfering substances and improve control of sample volume. It is well known in the art that electrode surfaces exposed to a wide range of biological molecules will suffer poisoning of catalytic activity and failure. However, utilizing the layers and membranes of the present invention, the active electrochemical surfaces of the sensor electrodes are preserved, allowing activity to be retained for extended periods of time *in vivo*. By limiting exposure of the platinum sensor surface to certain molecular species (e.g., molecules having a molecular weight below 34 Daltons, the molecular weight of hydrogen peroxide), *in vivo* sensor operating life in excess of one year in canine subjects has been observed.

A. <u>Angiogenic Layer</u>

[0076] For implantable glucose monitoring devices, a sensor/tissue interface must be created which provides the sensor with oxygen and glucose concentrations comparable to that normally available to tissue comprised of living cells. Absent such an interface, the sensor is

associated with unstable and chaotic performance indicating that inadequate oxygen and/or glucose are reaching the sensor. The development of interfaces in other contexts has been reported. For example, investigators have developed techniques that stimulate and maintain blood vessels inside a FBC to provide for the demanding oxygen needs of pancreatic islets within an implanted membrane. [*See, e.g.*, Brauker *et al.*, J. Biomed. Mater. Res. (1995) 29:1517-1524]. These techniques depend, in part, on the use of a vascularizing layer on the exterior of the implanted membrane. However, previously described implantable analyte-monitoring devices have not been able to successfully maintain sufficient blood flow to the sensor interface.

[0077] As described above, the outermost layer of the electrode-membrane region includes an angiogenic material. The angiogenic layer of the devices of the present invention may be constructed of membrane materials such as hydrophilic polyvinylidene fluoride (*e.g.*, Durapore®; Millipore Bedford, MA), mixed cellulose esters (*e.g.*, MF; Millipore Bedford, MA), polyvinyl chloride (*e.g.*, PVC; Millipore Bedford, MA), and other polymers including, but not limited to, polypropylene, polysulphone, and polymethylmethacrylate. Preferably, the thickness of the angiogenic layer is about 10 μm to about 20 μm. The angiogenic layer comprises pores sizes of about 0.5 μm to about 20 μm, and more preferably about 1.0 μm to about 10 μm, sizes that allow most substances to pass through, including, *e.g.*, macrophages. The preferred material is expanded PTFE of a thickness of about 15μm and pore sizes of about 5 μm to about 10 μm.

[0078] To further promote stable foreign body capsule structure without interfering with angiogenesis, an additional outermost layer of material comprised of a thin low-density non-

woven polyester (*e.g.*, manufactured by Reemay) can be laminated over the preferred PTFE described above. In preferred embodiments, the thickness of this layer is about 120 μm. This additional thin layer of material does not interfere with angiogenesis and enhances the manufacturability of the angiogenic layer. [*See* U.S. Pat. No. 5,741,330 to Brauker *et al.*, hereby incorporated by reference; also 5,782,912, 5,800,529, 5,882,354 5,964,804 assigned to Baxter].

B. <u>Bioprotective Membrane</u>

[0079] The inflammatory response that initiates and sustains a FBC is associated with both advantages and disadvantages. Some inflammatory response is needed to create a new capillary bed in close proximity to the surface of the sensor in order to i) continuously deliver adequate oxygen and glucose and ii) create sufficient tissue ingrowth to anchor the implant and prevent motion artifact. On the other hand, inflammation is associated with invasion of tissue macrophages that have the ability to biodegrade many artificial biomaterials (some of which were, until recently, considered nonbiodegradable). When activated by a foreign body, tissue macrophages degranulate, releasing from their cytoplasmic myeloperoxidase system hypochlorite (bleach), H_2O_2 and other oxidant species. Both hypochlorite and H_2O_2 are known to break down a variety of polymers, including polyurethane, by a phenomenon referred to as environmental stress cracking. [Phillips *et al.*, J. Biomat. Appl., 3:202-227 (1988); Stokes, J. Biomat. Appl. 3:228-259 (1988)]. Indeed, environmental stress cracking has been shown to limit the lifetime and performance of an enzyme-active polyurethane membrane stretched over the tip of a glucose sensor. [Updike *et al.*, Am. Soc. Artificial Internal Organs, 40:157-163 (1994)].

[0080] Because both hypochlorite and H_2O_2 are short-lived chemical species *in vivo*, biodegradation will not occur if macrophages are kept a sufficient distance from the enzyme active membrane. The present invention contemplates the use of a bioprotective membrane that allows transport of glucose and oxygen but prevents the entry of inflammatory cells such as macrophages and foreign body giant cells. The bioprotective membrane is placed proximal to the angiogenic membrane. It may be simply placed adjacent to the angiogenic layer without adhering, or it may be attached with an adhesive material to the angiogenic layer, or it may be cast in place upon the angiogenic layer as described in Example 1. The devices of the present invention are not limited by the nature of the bioprotective layer. However, the bioprotective layer should be biostable for long periods of time (*e.g.*, several years); the present invention contemplates the use of polymers including, but not limited to, polyurethane, polypropylene, polysulphone, polytetrafluoroethylene (PTFE), and poly(ethylene terephthalate) (PET).

[0081] The bioprotective membrane and the angiogenic layer may be combined into a single bilayer membrane as more fully described in Example 1. The active angiogenic function of the combined membrane is based on the presentation of the ePTFE side of the membrane to the reactive cells of the foreign body capsule and further to the response of the tissue to the microstructure of the ePTFE. This bioprotective/angiogenic membrane is unique in that the membrane does not delaminate as has been observed with other commercially available membranes (see FIG. 4A as compared with FIG. 4B). This is desirable for an implantable device to assure accurate measurement of analyte over long periods of time. Although the physical structure of the ePTFE represents a preferred embodiment, many other combinations of materials that provide the same function as the membrane of Example 1 could be utilized. For example, the

ePTFE could be replaced by other fine fibrous materials. In particular, polymers such as spun polyolefin or non-organic materials such as mineral or glass fibers may be useful. Likewise, the polyurethane bioprotective layer of Example 1, which includes a biostable urethane and polyvinylpyrrolidone (PVP), could be replaced by polymers able to pass analyte while blocking macrophages and mechanically retaining the fine fibrous material presented to the reactive cells of the foreign body capsule.

C. Sensing Membrane

[0082] The present invention contemplates membranes impregnated with enzyme. It is not intended that the present invention be limited by the nature of the enzyme membrane. The sensing membrane of a preferred embodiment is depicted in FIG. 1C as being a single, homogeneous structure. However, in preferred embodiments, the sensing membrane includes a plurality of distinct layers. In a particularly preferred embodiment, the sensing membrane includes the following four layers (in succession from the bioprotective membrane to the layer most proximal to the electrodes): i) a resistance layer; ii) an enzyme layer; iii) an interference layer; and iv) an electrolyte layer.

Resistance Layer

[0083] There is a molar excess of glucose relative to the amount of oxygen in samples of blood. Indeed, for every free oxygen molecule in extracellular fluid, there are typically more than 100 glucose molecules present [Updike *et al.*, Diabetes Care 5:207-21(1982)]. However, an immobilized enzyme-based sensor using oxygen (O_2) as cofactor must be supplied with oxygen in non-rate-limiting excess in order to respond linearly to changes in glucose concentration while

not responding to changes in oxygen tension. More specifically, when a glucose-monitoring reaction is oxygen-limited, linearity is not achieved above minimal concentrations of glucose. Without a semipermeable membrane over the enzyme layer, linear response to glucose levels can be obtained only up to about 40 mg/dL; however, in a clinical setting, linear response to glucose levels are desirable up to at least about 500 mg/dL.

[0084] The resistance layer includes a semipermeable membrane that controls the flux of oxygen and glucose to the underlying enzyme layer (*i.e.*, limits the flux of glucose), rendering the necessary supply of oxygen in non-rate-limiting excess. As a result, the upper limit of linearity of glucose measurement is extended to a much higher value than that which could be achieved without the resistance layer. The devices of the present invention contemplate resistance layers comprising polymer membranes with oxygen-to-glucose permeability ratios of approximately 200:1; as a result, one-dimensional reactant diffusion is adequate to provide excess oxygen at all reasonable glucose and oxygen concentrations found in the subcutaneous matrix [Rhodes *et al.*, Anal. Chem., 66:1520-1529 (1994)].

[0085] In preferred embodiments, the resistance layer has a thickness of less than about 45 μm, more preferably in the range of about 15 to about 40 μm, and most preferably in the range of about 20 to about 35 μm.

[0086] The resistance layer is desirably constructed of a mixture of hydrophobic and hydrophilic polyurethanes.

Enzyme Layer

[0087] In addition to glucose oxidase, the present invention contemplates the use of a membrane layer impregnated with other oxidases, *e.g.*, galactose oxidase, uricase. For an enzyme-based electrochemical glucose sensor to perform well, the sensor's response must neither be limited by enzyme activity nor cofactor concentration. Because enzymes, including the very robust glucose oxidase, are subject to deactivation as a function of ambient conditions, this behavior needs to be accounted for in constructing sensors for long-term use.

[0088] Excess glucose oxidase loading is required for long sensor life. When excess glucose oxidase is used, up to 1.5 years of performance may be possible from the glucose-monitoring devices contemplated by the present invention.

[0089] In one preferred embodiment, the enzyme layer includes a polyurethane latex.

Interference Layer

[0090] The interference layer includes a thin, hydrophobic membrane that is non-swellable and restricts diffusion of low molecular weight species. The interference layer is permeable to relatively low molecular weight substances, such as hydrogen peroxide, but restricts the passage of higher molecular weight substances, including glucose and ascorbic acid. The interference layer serves to allow analytes and other substances that are to be measured by the electrodes to pass through, while preventing passage of other substances.

[0091] Preferred materials from which the interference layer can be made include polyurethanes. In one desired embodiment, the interference layer includes an aliphatic polyetherurethane.

[0092] The interference layer has a preferred thickness of less than about 5 μm, more preferably in the range of about 0.1 to about 5 μm and most preferably in the range of about 0.5 to about 3 μm. Thicker membranes also may be useful, but thinner membranes are preferred because they have a lower impact on the rate of diffusion of hydrogen peroxide from the enzyme membrane to the electrodes.

Electrolyte Layer

[0093] To ensure electrochemical reaction, the electrolyte layer comprises a semipermeable coating that maintains hydrophilicity at the electrode region of the sensor interface. The electrolyte layer enhances the stability of the interference layer of the present invention by protecting and supporting the membrane that makes up the interference layer. Furthermore, the electrolyte layer assists in stabilizing operation of the device by overcoming electrode start-up problems and drifting problems caused by inadequate electrolyte. The buffered electrolyte solution contained in the electrolyte layer also protects against pH-mediated damage that may result from the formation of a large pH gradient between the hydrophobic interference layer and the electrode (or electrodes) due to the electrochemical activity of the electrode.

[0094] Preferably, the coating includes a flexible, water-swellable, substantially solid gel-like film having a "dry film" thickness of about 2.5 μm to about 12.5 μm, preferably about

6.0 µm. "Dry film" thickness refers to the thickness of a cured film cast from a coating formulation onto the surface of the membrane by standard coating techniques. The coating formulation includes a premix of film-forming polymers and a crosslinking agent and is curable upon the application of moderate heat.

[0095] Suitable coatings are formed of a curable copolymer of a urethane polymer and a hydrophilic film-forming polymer. Particularly preferred coatings are formed of a polyurethane polymer having anionic carboxylate functional groups and non-ionic hydrophilic polyether segments, which is crosslinked in the present of polyvinylpyrrolidone and cured at a moderate temperature of about 50°C.

[0096] Particularly suitable for this purpose are aqueous dispersions of fully reacted colloidal polyurethane polymers having cross-linkable carboxyl functionality (*e.g.*, BAYBOND®; Mobay Corporation, Pittsburgh, PA). These polymers are supplied in dispersion grades having a polycarbonate-polyurethane backbone containing carboxylate groups identified as XW-121 and XW-123; and a polyester-polyurethane backbone containing carboxylate groups, identified as XW-110-2. Particularly preferred is BAYBOND® 123, an aqueous anionic dispersion of an aliphate polycarbonate urethane polymer, sold as a 35 weight percent solution in water and co-solvent N-methyl-2-pyrrolidone.

[0097] Polyvinylpyrrolidone is also particularly preferred as a hydrophilic water-soluble polymer and is available commercially in a range of viscosity grades and average molecular weights ranging from about 18,000 to about 500,000, under the PVP K® homopolymer series by

BASF Wyandotte (Parsippany, NJ) and by GAF Corporation (New York, NY). Particularly preferred is the homopolymer having an average molecular weight of about 360,000, identified as PVP-K90 (BASF Wyandotte). Also suitable are hydrophilic, film-forming copolymers of N-vinylpyrrolidone, such as a copolymer of N-vinylpyrrolidone and vinyl acetate, a copolymer of N-vinylpyrrolidone, ethylmethacrylate and methacrylic acid monomers, and the like.

[0098] The polyurethane polymer is crosslinked in the presence of the polyvinylpyrrolidone by preparing a premix of the polymers and adding a cross-linking agent just prior to the production of the membrane. Suitable cross-linking agents can be carbodiimides, epoxides and melamine/formaldehyde resins. Carbodiimide is preferred, and a preferred carbodiimide crosslinker is UCARLNK® XL-25 (Union Carbide, Chicago, IL).

[0099] The flexibility and hardness of the coating can be varied as desired by varying the dry weight solids of the components in the coating formulation. The term "dry weight solids" refers to the dry weight percent based on the total coating composition after the time the crosslinker is included. A preferred useful coating formulation can contain about 6 to about 20 dry weight percent, preferably about 8 dry weight percent, of polyvinylpyrrolidone; about 3 to about 10 dry weight percent, preferably about 5 dry weight percent of cross-linking agent; and about 70 to about 91 weight percent, preferably about 87 weight percent of a polyurethane polymer, preferably a polycarbonate-polyurethane polymer. The reaction product of such a coating formulation is referred to herein as a water-swellable cross-linked matrix of polyurethane and PVP.

D. The Electrolyte Phase

[0100] The electrolyte phase is a free-fluid phase including a solution containing at least one compound, usually a soluble chloride salt that conducts electric current. The electrolyte phase flows over the electrodes (*see* FIG. 1C) and is in contact with the electrolyte layer of the enzyme membrane. The devices of the present invention contemplate the use of any suitable electrolyte solution, including standard, commercially available solutions.

[0101] Generally speaking, the electrolyte phase should have the same or less osmotic pressure than the sample being analyzed. In preferred embodiments of the present invention, the electrolyte phase includes saline.

E. The Electrode

[0102] The electrode assembly of this invention may also be used in the manner commonly employed in the making of amperometric measurements. The interstitial fluids containing the analyte to be measured is in contact with a reference electrode, *e.g.*, silver/silver-chloride, and the anode and cathode of this invention, which are preferably formed of platinum. In the preferred embodiment, the electrodes are connected to a circuit board in the body of the sensor, the current is read and the information is radiotransmitted to a receiver. The invention is not limited to this preferred embodiment. Indeed the membranes of the present invention could be used with any form of implantable sensor and adapted to the particular features of the sensor by one skilled in the art.

[0103] The ability of the present device electrode assembly to accurately measure the concentration of substances such as glucose over a broad range of concentrations enables the rapid and accurate determination of the concentration of those substances. That information can be employed in the study and control of metabolic disorders including diabetes.

IV. Sensor Implantation and Radiotelemetric Output

[0104] Long-term sensor performance is best achieved, and transcutaneous bacterial infection is eliminated, with implanted devices capable of radiotelemetric output. The present invention contemplates the use of radiotelemetry to provide data regarding blood glucose levels, trends, and the like. The term "radiotelemetry" refers to the transmission by radio waves of the data recorded by the implanted device to an *ex vivo* recording station (*e.g.*, a computer), where the data is recorded and, if desired, further processed.

[0105] Although totally implanted glucose sensors of three month lifetime, with radiotelemetric output, have been tested in animal models at intravenous sites [*see, e.g.* Armour *et al.*, Diabetes, 39:1519-1526 (1990)], subcutaneous implantation is the preferred mode of implantation [*see, e.g.*, Gilligan *et al.*, Diabetes Care 17:882-887 (1994)]. The subcutaneous site has the advantage of lowering the risk for thrombophlebitis with hematogenous spread of infection and also lowers the risk of venous thrombosis with pulmonary embolism. In addition, subcutaneous placement is technically easier and more cost-effective than intravenous placement, as it may be performed under local anesthesia by a non-surgeon health care provider in an outpatient setting.

[0106] Preferably, the radiotelemetry devices contemplated for use in conjunction with the present invention possess features including small package size, adequate battery life, acceptable noise-free transmission range, freedom from electrical interference, and easy data collection and processing. Radiotelemetry provides several advantages, one of the most important of which is the ability of an implanted device to measure analyte levels in a sealed-off, sterile environment.

[0107] The present invention is not limited by the nature of the radiotelemetry equipment or methods for its use. Indeed, commercially available equipment can be modified for use with the devices of the present invention (*e.g.*, devices manufactured by Data Sciences). Similarly, custom-designed radiotelemetry devices like those reported in the literature can be used in conjunction with the implantable analyte-measuring devices of the present invention [*see, e.g.*, McKean and Gough, IEEE Trans. Biomed. Eng. 35:526-532 (1988); Shichiri *et al.*, Diabetes Care 9:298-301 (1986); and Shults *et al.*, IEEE Trans. Biomed. Eng. 41:937-942 (1994)]. In a preferred embodiment, transmitters are programmed with an external magnet to transmit at 0.5 or 5-minute intervals, depending on the need of the subject; presently, battery lifetimes at transmission intervals of 5 minutes are approximately up to 1.5 years.

V. Experimental

[0108] The following examples serve to illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

[0109] In the preceding description and the experimental disclosure which follows, the following abbreviations apply: Eq and Eqs (equivalents); mEq (milliequivalents); M (molar); mM (millimolar) μM (micromolar); N (Normal); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); μg (micrograms); Kg (kilograms); L (liters); mL (milliliters); dL (deciliters); μL(microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); h and hr (hours); min. (minutes); s and sec. (seconds); °C (degrees Centigrade); Astor Wax (Titusville, PA); BASF Wyandotte Corporation (Parsippany, NJ); Data Sciences, Inc. (St. Paul, MN); DuPont (DuPont Co., Wilmington, DE); Exxon Chemical (Houston, TX); GAF Corporation (New York, NY); Markwell Medical (Racine, WI); Meadox Medical, Inc. (Oakland, NJ); Mobay (Mobay Corporation, Pittsburgh, PA); Sandoz (East Hanover, NJ); and Union Carbide (Union Carbide Corporation; Chicago, IL).

Example 1
Preparation of Composite Membrane of the Present Invention

[0110] The angiogenic layer may be an ePTFE filtration membrane (Zefluor™, 3.0μm P5PI001, Pall Gelman, Ann Arbor, MI) and the bioprotective membrane (C30P) may then be coated on the angiogenic layer. For example, the C30P coating solution was prepared by placing approximately 706 gm of dimethylacetamide (DMAC) into a 3L stainless steel bowl to which a polycarbonateurethane solution (1325 g, Chronoflex AR 25% solids in DMAC and 5100 cp) and polyvinylpyrrolidone (125 g, Plasdone K-90D) were added. The bowl was then fitted to a planetary mixer with a paddle type blade and the contents were stirred for 1 hour at room temperature. This solution was then coated on the ePTFE filtration membrane by knife-edge drawn at a gap of 0.006" and dried at 60°C for 24 hours.

[0111] Alternatively, the C30P solution prepared above can be coated onto a PET release liner using a knife over roll coating machine. This material is then dried at 305°F for approximately 2 minutes. Next, the Zefluor™ is immersed in 50:50 (w/v) mixture of tetrahydrofuran/DMAC and then placed upon the coated polyurethane polyvinylpyrrolidone material. Light pressure atop the assembly intimately embeds the ePTFE into the C30P layer. The membrane is then dried at 60°C for 24 hours.

Example 2
Preparation of the Sensing Membrane

[0112] The sensing membrane includes a resistance layer, an enzyme layer, an interference layer and an electrolyte layer. The resistance layer was prepared by placing approximately 281 gm of DMAC into a 3 L stainless steel bowl to which a solution of polyetherurethaneurea (344 gm of Chronothane H, 29,750 cp at 25% solids in DMAC) was added. To this mixture was added another polyetherurethaneurea (312 gm, Chronothane 1020, 6275 cp at 25% solids in DMAC). The bowl was fitted to a planetary mixer with a paddle type blade and the contents were stirred for 30 minutes at room temperature. The resistance layer coating solution produced is coated onto a PET release liner (Douglas Hansen Co., Inc. Minneapolis, MN) using a knife over roll set at a 0.012" gap. This film is then dried at 305°F.

[0113] The enzyme layer was prepared by placing 304 gm polyurethane latex (Bayhydrol 140AQ, Bayer, Pittsburgh, PA) into a 3 L stainless steel bowl to which 51 gm of pyrogen free water and 5.85 gm of glucose oxidase (Sigma type VII from Aspergillus niger) is added. The bowl was then fitted to a planetary mixer with a whisk type blade and the mixture was stirred for 15 minutes. Approximately 24 hr prior to coating, a solution of glutaraldehyde (15.4 ml of a

2.5% solution in pyrogen free water) and 14 ml of pyrogen free water was added to the mixture. The solution was mixed by inverting a capped glass bottle by hand for about 3 minutes at room temperature. This mixture was then coated over the resistance layer with a #10 Mayer rod and dried above room temperature preferably at about 50°C.

[0114] The interference layer was prepared by placing 187 gm of tetrahydrofuran into a 500 ml glass bottle to which an 18.7 gm aliphatic polyetherurethane (Tecoflex SG-85A, Thermedics Inc., Woburn, MA) was added. The bottle was placed onto a roller at approximately 3 rpm within an oven set at 37°C. The mixture was allowed to roll for 24 hr. This mixture was coated over the dried enzyme layer using a flexible knife and dried above room temperature, preferably at about 50°C.

[0115] The electrolyte layer was prepared by placing 388 gm of polyurethane latex (Bayhydrol 123, Bayer, Pittsburgh, PA in a 3 L stainless steel bowl to which 125 gm of pyrogen free water and 12.5 gm polyvinylpyrrolidone (Plasdone K-90D) was added. The bowl was then fitted to a planetary mixer with a paddle type blade and stirred for 1 hr at room temperature. Within 30 minutes of coating, approximately 13.1 ml of carbodiimide (UCARLNK) was added and the solution was mixed by inverting a capped polyethylene jar by hand for about 3 min at room temperature. This mixture was coated over the dried interference layer with a #10 Mayer rod and dried above room temperature preferably at about 50°C.

[0116] In order to affix this multi-region membrane to a sensor head, it is first placed into phosphate buffer (pH 7.4) for about 2 minutes. It is then stretched over the nonconductive body of sensor head and affixed into place with an o-ring.

Example 3
***In vivo* evaluation of glucose measuring devices including the biointerface membranes of the present invention**

[0117] *In vivo* sensor function was determined by correlating the sensor output to blood glucose values derived from an external blood glucose meter. We have found that non-diabetic dogs do not experience rapid blood glucose changes, even after ingestion of a high sugar meal. Thus, a 10% dextrose solution was infused into the sensor-implanted dog. A second catheter is placed in the opposite leg for the purpose of blood collection. The implanted sensor was programmed to transmit at 30-second intervals using a pulsed electromagnet. A dextrose solution was infused at a rate of 9.3 ml/minute for the first 25 minutes, 3.5 ml/minute for the next 20 minutes, 1.5 ml/minute for the next 20 minutes, and then the infusion pump was powered off. Blood glucose values were measured in duplicate every five minutes on a blood glucose meter (LXN Inc., San Diego, CA) for the duration of the study. A computer collected the sensor output. The data was then compiled and graphed in a spreadsheet, time aligned, and time shifted until an optimal R-squared value was achieved. The R-squared value reflects how well the sensor tracks with the blood glucose values.

[0118] To test the importance of the composite membrane of the invention described in Example 1, implantable glucose sensors including the composite and sensing membranes of the present invention were implanted into dogs in the subcutaneous tissues and monitored for

glucose response on a weekly basis. Control devices including only a bioprotective C30P layer ("Control") were compared with devices including both a bioprotective and an angiogenic layer ("Test"), which corresponded to the composite bioprotective/angiogenic membrane of the device of the present invention described in Example 1.

[0119] Four devices from each condition were implanted subcutaneously in the ventral abdomen of normal dogs. On a weekly basis, the dogs were infused with glucose as described above in order to increase their blood glucose levels from about 120 mg/dl to about 300 mg/dl. Blood glucose values were determined with a LXN blood glucose meter at 5-minute intervals. Sensor values were transmitted at 0.5-minute intervals. Regression analysis was done between blood glucose values and the nearest sensor value within one minute. Devices with an R-squared value greater than 0.5 were considered functional. Figure 3 shows, for each condition, the cumulative number of functional devices over the 12-week period of the study. The Test devices performed better than the Control devices over the entire 12 weeks of the study. All of the test devices were functional by week 8. In contrast, none of the control devices were functional until week 10, after which 2 were functional for the remaining 2 weeks. The data shows that the use of the inventive biointerface membrane enables the function of implantable glucose sensors.

[0120] The description and experimental materials presented above are intended to be illustrative of the present invention while not limiting the scope thereof. It will be apparent to those skilled in the art that variations and modifications can be made without departing from the spirit and scope of the present invention.